

Seed Vigour In Relation To Heat Sensitivity And Heat Resistance In Barley Evaluated By Multivariate Data Analysis

Birthe Møller^{1,2} and Lars Munck¹

ABSTRACT

J. Inst. Brew. 108(3), 286–293, 2002

The vigour loss model based on normal distribution after artificially ageing by heat treatment of barley seeds developed by Ellis and Roberts and further developed at Carlsberg by Aastrup et al. introducing vigour potential (VP), does not adequately describe seed vigour for all barley samples. In a preliminary investigation we have identified heat-resistant barley samples. In this investigation we found untreated barley samples from the field where heat treatment as high as 68°C for 4 h at 12% water content only decreases germination from 99.0% to 93.8% compared with 94.8% to 0.0% for some of the heat-sensitive barleys following the above mentioned model. The correlation between germination velocity measured by the germination index (GI) of untreated samples and VP is not consistent when comparing different barley material. It is concluded that the classic vigour loss model for heat treatment may be used as a worst case prediction for germination, but it does not address the variation found in practice, including the possible advantage of exploiting the naturally occurring heat resistance.

Key words: Barley, heat resistance, heat treatment, principal component analysis, vigour.

INTRODUCTION

The main requirement for malting barley is a complete, even and preferably rapid germination; that is, the barley should possess a high vigour. Riis and Bang-Olsen¹⁹ found that slow-germinating low-vigour barley which had an end germination percentage comparable to fast-germinating high-vigour barley demanded up to 42% longer malting time.

Moreover, vigour is important in the grain industry that produces seed for farmers for sowing, because low-vigour seeds will give a reduced grain yield, especially under stressed weather conditions⁸. A reduced vigour can affect the plant emergence in the field, so that the crop is not successfully established. In extreme cases, it is not possible to compensate for this effect by using more seed.

Furthermore, plants from low-vigour seeds do not achieve as high yield per plant as plants from high-vigour seeds⁸.

All these conditions may influence the production costs and the quality of the end product – barley for malting.

The circumstances that influence the ageing process have been investigated by several authors in many different experiments where methods to determine loss of vigour have been developed. These methods can roughly be divided into three categories: 1) tests monitoring biochemical changes, 2) germination tests where germination time and homogeneity are investigated, and 3) stress tests where seeds are exposed to a stressful environment, either prior to imbibition or during germination. Vigour tests included in this category are accelerated ageing by heat treatment, cool germination, controlled deterioration and the Hiltner test using Ziegel gravel²³.

With regard to heat treatment as a stress source, many theories have been proposed to exploit heat treatment experimentally in order to accelerate the ageing process.

Survival of barley with constant moisture content was studied after varying periods of heat treatment at 50–100°C in closed containers. Life duration was defined as a chemical reaction according to the equation of Arrhenius⁹. The temperature coefficient Q10 was determined as the factor by which the death rate of seed increases when temperature increases 10°C.

Groves¹⁴ studied the connection between life duration after heat treatment at different moisture contents and the temperature coefficient Q10 for ageing, but did not find a clear relationship.

Robertson et al.²² demonstrated that the decrease in germination percentage due to heating was dependent on water content (relative humidity). Kernel age and storage condition before testing also influenced artificial ageing. They suggested that if one knew how much humidity the grain could tolerate at a certain time and temperature, it would be possible to use this information as a guide in the choice of storage conditions. Robertson et al. were the first to propose the possibility of using temperature, humidity and germination percentage in a model to determine vigour²².

Robertson's theory expressed in words influenced the creation of the mathematical models of Roberts and Ellis.

Roberts²¹ assumed that ageing in a seed sample adhered to a normal distribution model, and saw the ageing process as a sigmoid curve. He described the relationship between the half-vital-period (the time it takes to kill 50% of the

¹The Royal Veterinary and Agricultural University, Dairy and Food Science, Food Technology, Rolighedsvej 30, Dk-1958 Frederiksberg C

²Corresponding author: E-mail: bm@kvl.dk

kernels) and the temperature and water content, and found a linear correlation²¹.

A better correlation was found between predicted and measured values of loss of vigour, when water content (%) was transformed to logarithmic values⁹. The earlier equations were written together resulting in Ellis and Roberts' vigour equation¹¹.

$$V = K_i - p/10^{(K_e - C_w * \log m - C_h * t - C_q)}$$

V = probit % germination, K_i = probit % germination at beginning of storage, p = storage time in days, $K_e = 9.983$, $C_w = 5.896$, $C_h = 0.04$, $C_q = 0.000428$, m = moisture content, t = temperature

The values of the constants were determined from "survival curves" for a sample of the barley variety Proctor stored in 52 different environments from -20 to 90°C with moisture contents between 5 and 25%¹⁰.

Based on the theoretical and empirical vigour model of Ellis and Roberts¹¹, Aastrup et al.¹ at Carlsberg defined the vigour potential (VP) in probits (probability scale), also precluding that germination follows a sigmoid curve based on the assumption of normal distribution of ageing with storage time¹. The life story of an ideal barley sample can thus be described as a decrease in dormancy followed by complete germination ending with the ageing process, which will result in loss of vigour. When the percentage of germination is transformed in a probability scale to probit (e.g. VP 3 = 99.9%, VP 0 = 50%, VP -1 = 15%), a straight line will occur, and VP can be determined as the intersection with the y-axis¹.

A barley lot will often contain a few grains that are dead due to mechanical damage and not because of loss of vigour. For this reason Aastrup et al.¹ introduced P_n, as the part of the normally distributed barley population, eliminating the effect of grain damage in the equation.

Two barley varieties, one original sample of each, were used by Aastrup et al.¹ to test the model, where 5 subsamples of each sample/variety were artificially aged for varying periods of time at 60°C at a water content of 12%.

It was assumed that accelerated ageing through heat treatment was indicative of decrease of vigour during long-term storage, which occurs in practice. There have been few publications regarding vigour potential since 1989. Apparently, the VP model is little used in practice.

Instead, the germination index (GI) determined on unheated seeds defined by Riis and Bang-Olsen¹⁹ was adopted as a measure of vigour, and seems to be more widely used.

In the following we will investigate vigour as a function of heat treatment on a wide range of barley varieties from different harvest conditions. The material will be studied with and without accelerated ageing by heat treatment expressed as germination percentage, vigour potential and germination index using multivariate data analysis for evaluation of the whole germination curves.

MATERIALS AND METHODS

Plant material

All samples were stored at 4°C before analysing.

A. Barley samples from 1993 with natural differences in vigour: 21 samples of Alexis, Ariel, Blenheim, Etna and Meltan varieties were used. The samples were

grown in different farm locations in Southern Sweden collected and received from Skånska Lantmännen, Malmö after harvest 1993.

B. Barley samples from harvest 1993 with artificial differences in vigour due to heat storage: Alexis, Carula, Etna and Lysimax varieties were used. The 4 samples, which displayed initial germination percentage of 97.3 to 98.5, were harvested in 1993 and artificially aged by high temperature storage at 58°C (See Heat Treatment) yielding 4 samples of Alexis (0, 10, 30, 40 h), 3 samples of Carula (0, 10, 30 h), 5 samples of Etna (0, 10, 30, 40, 50 h) and 3 samples of Lysimax (0, 10, 30 h).

C. Barley from harvest 1994 with natural differences in vigour: Samples from A and B were grown in 1994 in Southern Sweden and in Denmark on the islands of Zealand and Funen. 14 samples of Alexis, Blenheim, Lysimax and Meltan varieties were chosen to determine VP.

Weather conditions 1993 and 1994

Seed vigour is heavily dependent on weather conditions and research on vigour under field conditions is severely hampered by the fact that only one to two years out of ten show drastic effects on germination.

In 1993 the spring and early summer in Southern Scandinavia were characterised by rain deficit. From sowing in March to July it only rained 50% of the average amount. July was colder than normal and had 16% fewer hours of sun than normal. However, July had 50% more rain than usual, most of it in the last two weeks of the month. In August the precipitation was normal, although most of the rain fell in the two first weeks of the month⁶. Thus, the humidity conditions made the harvest in 1993 quite difficult, and most of the grain was harvested with more than 15% moisture content, causing in some cases severe losses in germination capacity.

The spring (March) of 1994 was wet with 100% more rain than the normal amount. Therefore, the sowing of the experiments was delayed until late April. In June there was 20% more rain than usual. July was dry and hot, and August had normal precipitation, but most of it fell in late August after the barley was harvested⁷. This resulted in dry and good quality seed.

It is concluded that 1993 and 1994 were contrasting years with regard to the effect of weather conditions on harvest conditions and germination of the produced seeds.

Germination analysis

Two different germination analyses were made: (1) to determine total germination percentage and germination index (GI) after harvest using a H₂O₂ solution to remove dormancy, and (2) to determine the percentage of germinated kernels after heat treatment, after which the vigour potential (VP) was calculated. Petri dishes and water were used for this analysis (see below). In both analyses the samples were placed in a dark Refritherm incubator at 20°C. Four replicates were made for every sample and the standard deviation between the replicates was less than 5%.

1. Germination Index (GI)¹⁹: 4 × 100 kernels were steeped and germinated in a beaker with 50 mL 0.75% H₂O₂ solution added for three days. Percentage of germinated kernels (n) was calculated and removed after 24, 48 and 72 h. Every day the H₂O₂ solution was changed and

new was added to each sample. This test was carried out on samples immediately after harvest. Germination Index (GI) was calculated according to the following equation¹⁹:

$$GI = \frac{10 \times (n24 + n48 + n72)}{n24 + (n48 \times 2) + (n72 \times 3)}$$

n = % germinated kernels after 24, 48 and 72 h of germination

2. Vigour Potential (VP)¹: 4 × 100 kernels were germinated in 90 mm petri dishes with two layers of filter paper (Whatman No. 1) and 5 mL H₂O for 8 days at 20°C. Germinated kernels were counted and removed every third day. This was carried out at least two months after harvest; so most dormancy was broken.

Heat treatment

Heat treatments were carried out with two different purposes:

1. To produce artificially aged samples by heat storage for comparison with naturally aged samples: The 4 samples, Alexis, Carula, Etna and Lysimax, all contained 12% water. Ageing (loss of vigour) was achieved by heat treatment of barley lots in watertight plastic bags in a water bath at 58°C for 0-50 h. Subsamples were removed every 10 h. These samples were analysed in order to determine VP and included in the field experiment in 1994.

2. To determine vigour potential (VP) according to Aastrup et al.¹: Vigour potential was determined by heat-treating 10 subsamples of every barley lot at 12% water content contained in welded plastic aluminium bags in a water bath at 68°C for 0-4½ h, where subsamples were removed every 30 min. After this treatment every subsample was germinated according to germination method 2. The results from 8 days of germination were plotted, yielding a germination curve dependent on heat treatment from which the vigour potential was calculated using the Carlsberg vigour model¹.

If the moisture content in a sample was too high, the sample was spread out in a thin layer at room temperature until the moisture content was below 12%. The samples were corrected to 12.0% water content by the addition of the missing volume of water. The sample was then shaken

and left for 24 h in a closed plastic box, after which the moisture content was checked.

Chemical analysis

Moisture content was determined according to ICC 110/1³.

Activity of α-amylase was determined according to ICC 108³.

Data analysis

Principal Component Analysis (PCA) was performed according to Martens and Næs¹⁵ using the software “Unscrambler” from CAMO ASA, Norway. The aim of the PCA algorithm is to determine the latent factors or principal components (PCs) in the data set, which describe most variation. Based on vector algebra the algorithm calculates and compresses the data material (whole germination curves after heat treatment) into scores for principal components, which are plotted on a score plot. The position of a sample in the score plot expresses the pattern of the corresponding germination curve, so those samples with similar scores reflect the same pattern. The variables involved (germination percentage (g) after heat treatment in e.g. 4½ h (g4½) and untreated (g0), expressed as loadings, can be plotted together with the scores in a biplot. A score for a germination curve placed near a loading indicates a high influence by the variable.

RESULTS

In Table IA the differences and ranges in vigour potential (VP), germination percentage and germination index (GI) of all the barley varieties naturally and artificially aged are shown. These results show that the germination index (GI) varies from 4.9 to 8.9 for the naturally aged samples and from 3.4 to 8.7 for the artificially aged samples. Three samples of naturally aged Alexis had a final germination percentage after 3 days (method 1) less than 90%, whereas 5 of the artificially aged samples by heat storage (Carula 30 h; Etna 30, 40, 50 h; Lysimax 30 h) had less than 90% germination. In the naturally aged samples the level of VP varies from 0.6 to 4.4, whereas in the arti-

TABLE IA. Min-Max values for vigour potential (VP), germination index (GI) and total germination percentage for the 50 samples. Min-Max values of α-amylase activity for 28 samples of the total.

Variety	Samples	VP	GI	Germ_tot	α-Amylase
Naturally aged 1993–1994					
Alexis	12	0.6–4.4	4.9–8.9	49.8–98.8	0.2–2.0 (n = 12)
Ariel	2	0.8–1.7	8.3–8.4	95.8–97.0	—
Blenheim	7	1.5–2.9	5.7–8.8	93.5–97.0	0.1–1.2 (n = 5)
Etna	2	0.9–2.1	5.0	95.8–97.5	—
Lysimax	2	2.6–2.9	6.8–6.9	98.8–99.5	0.1 (n = 2)
Meltan	10	1.2–3.1	5.2–7.3	97.0–99.3	0.1–0.7 (n = 8)
Artificially aged					
Alexis – untreated	1	1.0	8.2	98.5	0.4 (n = 1)
heat-treated 10-40 h	3	0.3–1.4	4.4–6.8	95.3–98.3	—
Carula – untreated	1	1.2	8.1	97.3	—
heat-treated 10-30 h	2	0.5–1.0	3.9–6.4	25.0–95.3	—
Etna – untreated	1	1.5	5.0	97.3	—
heat-treated 10-50 h	4	0.1–1.5	3.4–5.0	15.3–94.8	—
Lysimax – untreated	1	2.3	8.7	97.8	—
heat-treated 10-30 h	2	0.4–2.2	3.8–6.0	32.5–91.8	—

TABLE IB. Average value and variation for vigour potential (VP), germination index (GI) and % total germination in artificially aged samples, naturally aged samples in total and for the two separate years.

	n	VP	GI	Germ.%
Artificially aged	11	0.9 ± 0.8	4.8 ± 1.2	73.3 ± 16.2
Naturally aged	35	2.0 ± 0.8	6.7 ± 1.2	94.2 ± 4.8
Naturally aged 1993	21	1.8 ± 0.8	6.6 ± 1.3	93.0 ± 5.9
Naturally aged 1994	14	2.4 ± 0.4	6.7 ± 1.2	96.4 ± 1.3

ficially aged samples the level of VP varies from -0.5 to 2.2, which is markedly lower. Alexis is an exception here, because the given heat treatments for 10 and 30 h apparently have higher VP (VP = 1.4 and 1.3) than the untreated sample (VP = 1.0). The reason for this will be discussed later.

A comparison of the average values of the artificially and naturally aged samples in Table IB shows that the naturally aged samples have higher values of VP, GI and total germination compared to the artificially aged samples produced by heat storage. It is furthermore seen that the naturally aged samples grown and harvested in 1994 have higher values of VP, GI and total germination compared to the naturally aged samples harvested in 1993.

The extreme germination curves (Fig. 1A) obtained by heat treatment at 68°C with 12% water content from ½-4½ h, were selected from the PCA classification plots (Fig. 2A). One group of samples followed the vigour model of Ellis and Roberts¹¹ with a strong decrease in germination after 1-2 h of heat treatment, resulting in reverse S-shaped curves as represented by No. 11 Alexis (Fig. 1A). No. 29 Meltan (Fig. 1A), displaying horizontal linear curves where germination exceeded 90% even after 4 h of heat treatment represents another group of apparently heat-resistant samples. The third group also showing horizontal curves, but on a low germination level already in the

untreated sample, is represented by the heat-stored sample No. 03 Carula.

In untreated barley samples from the field we found that germination after heat-treatment at 68°C for 4½ h in the extreme samples decreased from 99.0% to 93.8% in the heat-resistant samples compared with 94.8% to 0.0% for some of the heat-sensitive barleys following the above mentioned model.

The Carlsberg model¹ was used to calculate the vigour potential (VP) in a probit scale. As an example, the germination heat treatment curves from Fig. 1A are shown in Fig. 1B where the probit scale is used instead of percentage of germination. The germination curve plotted on probability paper follows approximately a straight line – the tangent of the curve, which is extrapolated to the y-axis where the intersection gives VP.

One would think that sample No. 29 should have the highest VP, because this sample was more resistant to the stress factor of heat compared with the other two samples. This is not the case with the Carlsberg model. As one can see in Fig. 1B, the heat-resistant sample No. 29 has a VP of 3.1, whereas the heat-sensitive sample No. 11 (following the VP model) has a VP of 4.4. The probit curves for the two samples have the same intersection at the Y-axis, but the intersection of the tangent for the two curves which defines VP makes a difference between the two samples according to the slope. The third sample (No. 03) has the lowest VP, as expected.

Principal component analysis (PCA) is used to represent the different patterns of the germination curves for the total number of barley samples (n = 50) (Fig. 2A). The PC 1 and PC 2 explain 76% and 14% of the variation respectively. The notations refer to variety, and behind every score point in the PCA is a corresponding germination curve, as shown in Fig. 1A. Samples lying close to each other in the plot have similar scores and patterns with re-

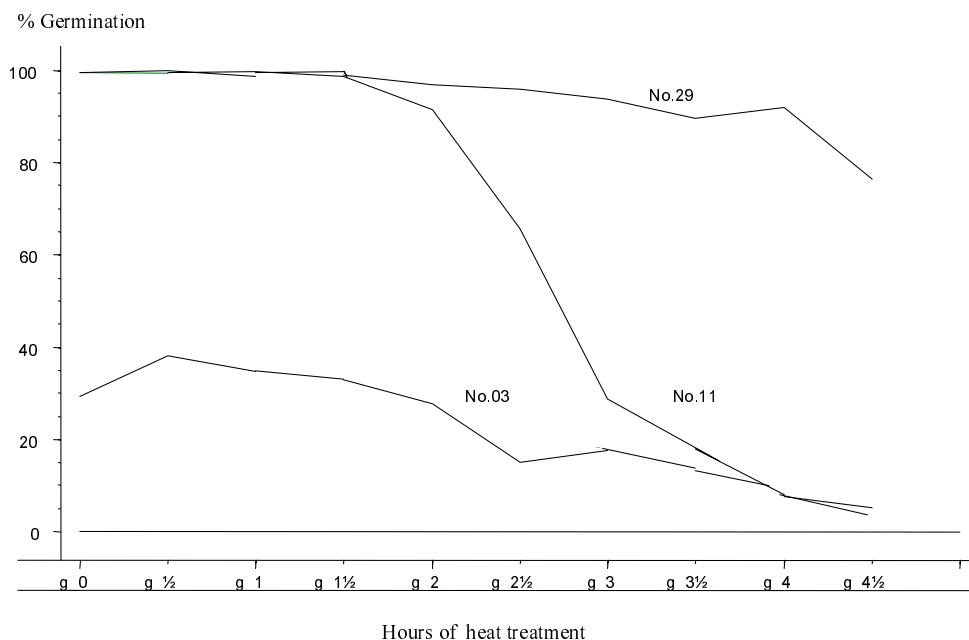


FIG. 1A. Germination curves for samples No. 03 (Carula, artificially aged 30 h), No. 11 (Alexis, naturally aged) and No. 29 (Meltan, naturally aged). Total germination percentage after heat-treatment at 68°C in 0-4½ h, where sub-samples are removed every ½ h.

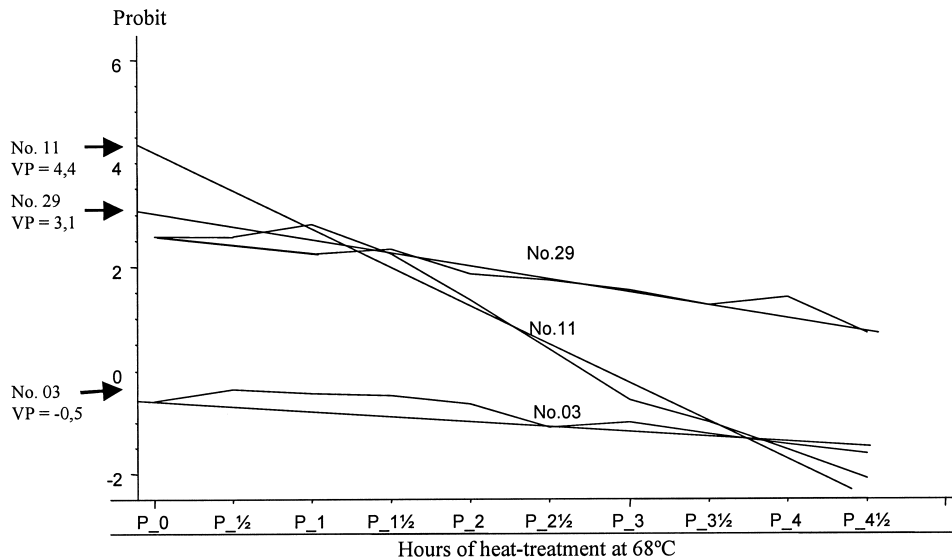


FIG. 1B. Germination curves for samples No. 03, 11 and 29. Total germination percentage in probit after heat treatment at 68°C in 0-4½ h where sub-samples are removed every ½ h. The points where the tangents meet the y-axis (arrows) is defined as the initial vigour potential (VP).

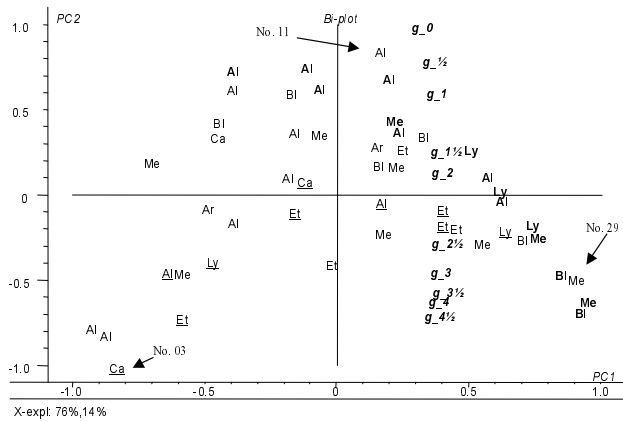


FIG. 2A. Biplot (PC1:PC2) made on germination curves for the heat treated samples harvested in 1993 and 1994. Al = Alexis, Ar = Ariel, BI = Blenheim, Ca = Carula, Et = Etna, Ly = Lysimax, Me = Meltan. Underlined samples are artificially aged with heat storage before the heat treatment procedure according to the Carlsberg model. Bold samples are harvested in 1994. All others are from 1993. Loadings: e.g. g_0 = total % germination in untreated sample and $g_{½}$ = heat treated ½ h at 68°C.

gard to the germination curve. As can be seen in the biplot, there is no systematic division between varieties or between naturally and artificially aged (underlined) samples (Fig. 2A). The corresponding loadings for the variables regarding germination after heat treatment ($g_0 - g_{4½}$) in the biplot in Fig. 2A reveal a systematic trend in the position of the variables ranging from germination percentage at 8 days for the unheated sample g_0 in the top right corner to the corresponding values for heat-treatment at 4½ h below. The long heat-treatment variables appear in the same corner as the heat-resistant samples like No. 29 (Fig. 2A).

In Fig. 2B the same plot (loadings not displayed) is shown as in Fig. 2A, although the notation refers to germi-

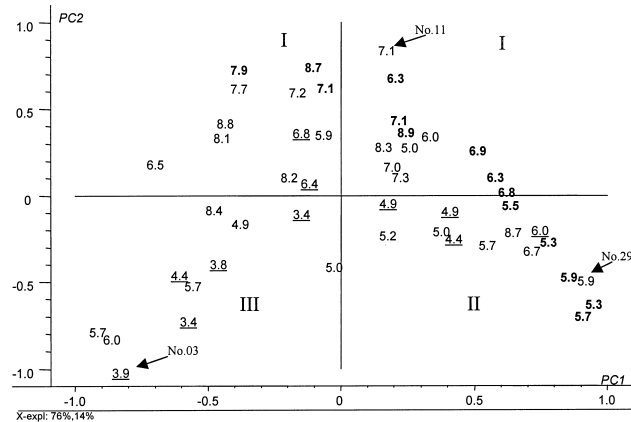


FIG. 2B. Plot with scores (PC1:PC2) made on germination curves for the 50 heat treated samples harvested in 1993 and 1994. The numbers refer to the germination index (GI) for every sample. Underlined samples are artificially aged before heat treatment according to the Carlsberg model. Bold samples are harvested in 1994.

nation index (GI) for the samples instead of variety. It appears as if the samples in the upper half (Group I) have higher GI values than the samples in the lower half. Here there are two groups: one to the bottom left containing samples with low GI (Group III) and one to the bottom right containing samples with intermediate GI values (Group II). The three examples of germination curves No. 11, No. 29 and No. 3 illustrated in Fig. 1A are representatives for each of the three groups I, II and III, respectively. The samples with reverse S-formed curves following the vigour model are placed in the upper half of the score plot (Group I) with high values of GI. The samples with low initial germination percentages (Group III) are placed bottom left (lowest GI values), while the heat-resistant samples with high germination percentage in the untreated sample (as well as in the initially heat-stored samples) are

TABLE II. Average and 5% confidence interval for GI, germination percentage (8 days) of samples, untreated and heat treated at 68°C (12.0% H₂O) in 4½ h calculated for Group I, II and III. Different letters refer to significant differences (5% level) between groups. Activity of α-amylase for 28 samples divided in the three groups are shown as well.

	n	VP	G%untreat.	G%4½h	α-Amylase
Group I	24	7.2 ± 0.4 A	95.6 ± 2.0 A	17.9 ± 7.9 A	0.4 ± 0.3 (n = 18) A
Group II	15	5.7 ± 0.6 B	94.1 ± 3.5 A	53.6 ± 12.4 B	0.2 ± 0.1 (n = 6) A
Group III	11	5.0 ± 1.0 B	56.8 ± 11.2 B	12.3 ± 16.3 A	0.7 ± 0.9 (n = 4) A

placed bottom right (Group II), where the GI values are intermediate between the other two groups (Fig. 2B).

When average GI for the three groups is calculated, there is a clear statistical difference between the groups (Table II). The confidence interval (5% level) shows that there is significant difference between GI for Group I and the other groups, but there is no significant difference between GI for Groups II and III. Comparing the average germination percentage with the untreated samples for the three groups, it is seen that there is no significant difference between Group I (heat sensitive) and Group II (heat resistant). Group III however is significantly different from the two groups due to the low initial germination percentage of the samples belonging to this group. This picture changes when looking at the average germination percentage for the groups after 4½ h of heat treatment. Now there is a clear significant difference between Group I and Group II. The samples in Group III are defined as heat resistant because the decrease in germination percentage from untreated to 4½ h of heat treatment are low. However when the germination percentage is initially low, the germination percentage after heat treatment will also be low. Therefore Group III is not significantly different with respect to GI from Group I (Table II). It is concluded that group III is irrelevant for the malting industry due to its low initial germination percentage.

DISCUSSION

The samples grown and harvested in 1994 have higher average values of VP, GI and total germination percentage than the samples from 1993 (Table IB). This is as expected, because the weather conditions during harvest were almost optimal in 1994, but difficult in 1993. With regard to the heat resistance and heat sensitivity, it is not possible to see a systematic difference between the two years. In Figures 2A and B it is seen that samples from 1994 (in bold) are placed from the left top to the right bottom corner, showing both heat-sensitive and heat-resistant samples together with the samples from 1993.

From the example in Fig. 1B it is obvious that the model of vigour potential is not adequate to describe all barley samples. In some cases when samples show heat resistance, the VP will be lower for the sample resisting stress than for the heat-sensitive sample, which reacts to stress. Another example is the artificially aged samples of Alexis, which have higher vigour potentials compared to the untreated sample of Alexis (Table IA). This is due to the fact that the tangents for the two heat-stored samples have a larger slope than the unheated sample, resulting in a higher VP value compared to the untreated raw sample.

When comparing VP with germination index (GI), which is another potential indicator of vigour, the heat-

sensitive samples in this experiment have higher GI than the heat-resistant samples. Aastrup et al.² found a high correlation ($r = 0.95$ and $r = 0.92$) between VP and GI for two barley samples (varieties Ca108725 and Klages) divided in 10 subsamples, which were artificially aged by heat storage.

Table III shows the correlation coefficient (r) between VP and GI from our experiment. There is no significant correlation for the total material ($r = 0.33$). When the samples are divided into naturally and heat stored samples, there is no correlation for the naturally aged samples ($r = -0.09$), but there is a weak positive correlation between VP and GI for the heat stored samples ($r = 0.63$). When the heat-stored samples are divided into varieties and correlation coefficients are calculated, Alexis has a low correlation value ($r = 0.42$), but r is higher for Lysimax, Etna and Carula separately and combined (Lysimax, $r = 0.86$; Etna, $r = 0.86$; Carula, $r = 0.95$ and combined $r = 0.65$). It seems that the correlation between VP and GI in heat stored samples is dependent on variety and that samples that are artificially aged by heat storage have a relatively high correlation coefficient, confirming the results of Aastrup et al.². This result deviates from the naturally aged samples, which display a low correlation coefficient between VP and GI.

GI is determined after germination for three days whereas VP is determined after 8 days of germination. Germination for 8 days is not interesting for the malting industry, but 8 days was chosen to obtain the extreme criterium for vitality and to compare with earlier experiments².

TABLE III. Correlation between vigour potential (VP) and germination index (GI).

Correlation VP-GI	n	r
All samples	50	0.33
Group I	24	-0.07
Group II	15	0.4
Group III	11	0.33
Naturally aged	35 + 4	-0.09
Alexis	12 + 1	0.02
Ariel	2	—
Blenheim	7	-0.78
Etna	2 + 1	0
Lysimax	2 + 1	-0.84
Meltan	10	-0.18
Naturally aged 1993	21 + 4	0.01
Naturally aged 1994	14	-0.63
Artificially aged + untr. control	11 + 4	0.58
Artificially aged	11	0.63
Alexis	4	0.42
Carula	3	0.95
Etna	5	0.86
Lysimax	3	0.86
C+E+L	11	0.65

From our experiments it is demonstrated that the vigour model developed by Ellis and Roberts¹¹ using heat treatment and further developed with vigour potential at Carlsberg¹ is not adequate to classify and describe all barley samples. In addition to the sigmoid curve postulated by the statistical model^{1,11} there are at least two other extreme reaction models (Fig. 1A), both comparatively insensitive to heat treatment, but exhibiting high and low germination levels. There is a continuous variation between the three models, which is best detected and visualised by multivariate data analysis (PCA).

A review of the published literature^{1,9-13,20-22} shows that earlier experiments with heat treatment of barley were made with very few varieties producing sub-samples by heat treatment of a very limited number of original barley field lots which all were artificially aged. In order to test the vigour models of Ellis and Roberts¹¹ and Aastrup et al.¹ in our experiment we tested 50 barley samples from 7 different varieties grown in two different years and, in contrast to the other authors, included both artificially and naturally aged samples. Furthermore, we have succeeded in finding naturally aged samples with a broader range in VP than the artificially aged samples.

The results of the current study confirmed those of Riis and Bang-Olsen¹⁹ who performed experiments with heat-treated samples (artificially aged) for which GI was determined. The heat treatment was carried out on four different samples of different varieties: Alexis, Ariel, Triumph and Prisma. The samples reacted differently to the heat treatment, where Ariel in this experiment was most influenced by the heat treatment as opposed to Alexis that in this case was most resistant to the heat. All the samples had lower GI when they were heat-treated¹⁹.

Pre-germination has been shown by several authors to cause increased sensitivity with regard to storage^{4,5,18}.

In order to elucidate if the observed heat sensitivity and resistance in our experiment is related to pregermination or de novo produced enzymes, the activity of α -amylase was analysed on 28 of the 50 samples.

As can be seen from Table IA the α -amylase activity is generally low in our material. There is no indication of increased α -amylase activity with respect to either the heat sensitive or the heat resistant samples (See Table II).

The analyses show that the content of α -amylase differ from 0.1-2.0 units α -amylase. No correlations between content of α -amylase and e.g. vigour or heat sensitivity is found. As an example the content of α -amylase for the heat-sensitive sample "Alexis no. 11" is compared with the heat resistant "Meltan no. 29" (Fig. 1A and 1B). The sensitive sample has an activity of 0.3 units α -amylase whereas the heat-resistant is 0.2 units α -amylase.

CONCLUSIONS

We have found heat-sensitive samples following the vigour model^{1,11} as well as heat-resistant samples¹⁶ with more than 90% germination after heat treatment at 68°C for 4½ h, which do not follow the model. Heat-sensitive samples in our material have a tendency towards a faster germination and therefore a higher GI than heat-resistant samples.

We conclude that the above mentioned model for vigour as related to heat treatment may only be used as a worst case scenario for prediction of survival.

The genetic and environmental background for heat resistance of germination found in several barley samples in this investigation will be discussed in a subsequent publication¹⁷.

ACKNOWLEDGEMENTS

The Seed Company "Skånska Lantmännen" through the SL Foundation and the Danish Cereal Network are greatly thanked for financing this study. We are very grateful to Monica Frank and the rest of the staff at Agrolab, Eslöv for collecting and analysing the barley samples from the 1993 harvest according to germination capacity. We are also grateful to Gilda Kischinsky for her comments to the article.

REFERENCES

1. Aastrup, S., Riis P. and Hansen, J.R., High vigour – the basis for high malting barley quality. Proceedings of the European Brewing Convention Congress, Zurich, IRL Press: Oxford, 1989, 171-178.
2. Aastrup, S., Riis, P. and Munck, L., Controlled removal of dormancy renders prolonged storage of sprouting resistant malting barley. Fifth International Symposium on Pre-Harvest Sprouting in Cereals, Norway, Westview Press: Oxford, 1989, 329-337.
3. Anonymous, Standard methods of the International Association for Cereal Chemistry (ICC) ICC-standards, Verlag Moritz Schäfer: Detmold, 1998.
4. Bason, M.L., Ronalds, J.A. and Wrigley, C.W., Prediction of safe storage life for sound and weather-damaged malting barley. *Cereal Foods World* 1993, **38**, 361-363.
5. Carn, J.D., Alpha amylase indicates problems with malting of stored barley. *Food Technology in Australia*, 1982, **34**, 82-83.
6. DMI (Danish Meteorological Institute), *Weather in Denmark*, 1993.
7. DMI (Danish Meteorological Institute), *Weather in Denmark*, 1994.
8. Ellis, R.H., Seed and seedling vigour in relation to crop growth and yield. *Plant Growth Regulation*, 1992, **11**, 249-255.
9. Ellis, R.H. and Roberts, E.H., Improved equation for the prediction of seed longevity. *Annals of Botany*, 1980, **45**, 13-30.
10. Ellis, R.H. and Roberts, E.H., The influence of temperature and moisture on seed viability period in barley (*Hordeum distichum* L.). *Annals of Botany*, 1980, **45**, 31-37.
11. Ellis, R.H. and Roberts, E.H., The quantification of ageing and survival in orthodox seeds. *Seed Science and Technology*, 1981, **9**, 377-409.
12. Ellis, R.H. and Roberts, E.H., An investigation into the possible effects of ripeness and repeated threshing on barley seed longevity under six different storage environments. *Annals of Botany*, 1981, **48**, 93-96.
13. Goodspeed, T.H., The temperature coefficient of the duration of life of barley grains. *Botanical Gazette*, 1911, **51**, 220-224.
14. Groves, J.F., Temperature and life duration of seeds. *Botanical Gazette*, 1917, **58(3)**, 169-189.
15. Martens, H. and Næs, T., *Multivariate Calibration*, Wiley: New York, 1993, 35-72 and 97-100.
16. Møller, B. and Munck, L., Barley seed vitality in relation to heat-susceptibility and -resistance. The VII International Barley Genetics Symposium, University Extension Press: University of Saskatchewan, Saskatoon, Canada, 1996, 646-648.
17. Møller, B., Molina-Cano, J.L. and Munck, L., Variation in malting quality and heat resistance in the malting barley variety "Alexis". *Journal of the Institute of Brewing*, 2002, **108(3)**, 294-302.
18. Pitz, W.J., Rapid and objective methods for estimation of pre-

- germination and viability in barley. *Journal of the American Society of Brewing Chemists*, 1991, **49**(3), 119-127.
19. Riis, P. and Bang-Olsen, K., Breeding high vigour barley – a short cut to fast maltings. Proceedings of the European Brewing Convention Congress, Lisbon, IRL Press: Oxford, 1991, 101-108.
 20. Roberts, E.H. and Abdallah, F.H., The influence of temperature, moisture and oxygen on period of seed viability in barley, broad bean and peas. *Annals of Botany*, 1968, **32**, 97-117.
 21. Roberts, E.H., The viability of cereal seed in relation to temperature and moisture. *Annals of Botany*, 1960, **24**, 12-31.
 22. Robertson, D.W., Lute, A.M. and Gardner, R., Effect of relative humidity on viability, moisture content and respiration of wheat, oats and barley in storage. *Journal of Agricultural Research*, 1939, **59**, 281-291.
 23. Spears, J.F. An introduction to seed vigour testing. In: Seed Vigour Testing, Proceedings from 24th Congress of the International Seed Testing Association, H.A. van de Venter Ed., Copenhagen, 1995, 1-9.

(Manuscript accepted for publication April 2002)